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The overall objective is to determine the 3D structures of bacterial toxins and enterotoxins by the single crystal x-ray diffraction technique. Staphylococcal enterotoxin B (SEB) has been crystallized. X-ray diffraction data to 2.5 Å has been collected from native and derivative crystals. The crystal structure analysis is continuing. Attempts are underway to crystallize other enterotoxins.

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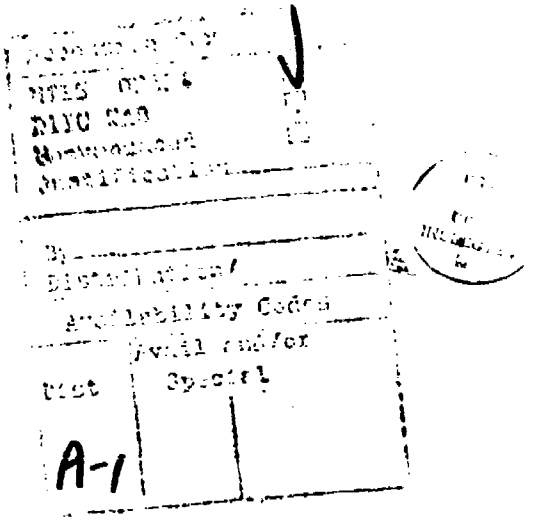
INTRODUCTION:

The overall objective of this research is to determine the three dimensional structures of some bacterial toxins which are prevalent in food poisoning. The structural data is crucial for understanding the mechanisms of the biological actions of these agents and for designing effective measures to counteract or to prevent the toxicity. During the first year of the study, the major effort was concentrated upon the determination of the crystal structure of SEB, since only for this specific enterotoxin were diffraction quality crystals on hand. Nevertheless, it was a good protein to begin the study with because extensive biochemical physiological, and immunological data had been published about it. The site of its centric, teratogenic, and immunological activities were shown to reside within certain broad limits of the amino acid sequence, which also is known. Crystallography was needed to show the spatial arrangements of the active sites, their proximities, and whether or not they overlap, in addition to resolving the detailed teritiary and secondary structures of the protein molecule.



BODY:

Single crystals of SEB which were suitable for crystal structure analysis were grown by methods which we published recently (1). X-ray data have been collected from the native protein and from heavy atom derivatives. A Nicolet-Xentronics area detector and a rotating anode x-ray source (12 KW) were used for the measurement of the diffracted intensities. The preliminary x-ray data were confusing in that the cell dimensions from different native crystals varied more than would have been expected. The problem was demonstrated to have been caused by the fact that the preparation which we had used was a mixture of three very similar toxins. They were separated by chromatofocusing into individual bands which could be crystallized. Later on better separation was attained with isoelectric focusing. Two of the three "isotoxins" were crystallized. The cell constants of the two forms differed by small, but statistically significant, amounts. Both forms crystallized in $P2_12_12_1$, however. Native data was collected to 2.7 A^o (Form I) and 2.3 A^o (form II). The cell constants are:

Form I: a = 45.31 b = 70.61 c = 78.12 A^o

Form II: a = 45.48 b = 68.30 c = 79.40 A^o

An extensive search for isomorphous heavy atom derivatives was started, and it is still going on. Only one, a $Pt(NH_3)_2Cl_2$ derivative, appears to be suitable for determining the phases. The 2.5A data were collected from this particular derivative. The R merged with native data is 10 percent. The Patterson map clearly shows three sites with occupancy factors of 1.00, 0.66, and 0.45. Refinement of the heavy atom positions gave an R centric of 0.43 for 6A data and 0.54 for 3A data. Potassium platinum thiocyanate gave a derivative with substitutions at the same three sites, but this time the data was too noisy to use in a structure analysis. We tried to make derivatives from the following: Mercuric iodide, potassium gold thiocyanate, thallium fluoride, sodium tungstate, p-chloromercuric phenyl sulfonic acid, dysprosium chloride and gadolinium chloride. None were satisfactory. Osmium ammonium chloride and iridic sodium chloride transformed form II crystals into form I. Gold potassium chloride and mercuric chloride appear to be promising derivatives, but further experimental work on them is required in order to see if we can find the conditions that yield useful derivatives.

We tried to crystallize a complex of SEB with a monoclonal antibody. This work is still in progress. Attempts to crystallize SEA and SEC also are underway, but our supply of SEC is short. Crystallization experiments were initiated on two other bacterial food poisoning toxins: CPE (Clostridium perfringens enterotoxin) and one of the botulism neurotoxins E). The clostridral enterotoxin experiments were carried out in the VAMC, while a small number of neurotoxin trials were made at Fort

Detrick by Dr. J. Schmidt. Both toxins proved to be refractory toward crystallization so far. The crystallization of an anti-CPE monoclonal antibody in a complex with CPE was attempted also. Very thin needles were obtained which are currently too small for a diffraction study.

Very significant to progress of this research was the acquisition of an area detector capable of measuring the diffracted intensitities with extreme efficiency. The process of assembling the equipment and of interfacing it to a computer is in progress.

The research on this project which was done this year was presented at the meeting of the American Crystallographic Association held in Philadelphia during 6/27/88 to 7/1/88 (2), and the preliminary x-ray data were published (1).

CONCLUSIONS:

(1) The crystal structure analysis of SEB is progressing steadily. We anticipate that data collection will continue, although the effort to solve the phase problem will begin shortly. Three possible methods are under consideration: SIRAS, ISIR, and the direct method with single isomorphous replacement.

(2) Work on the other toxins, described in our proposal, is continuing.

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ACA meeting, Philadelphia, June 27-July 1, 1988.